

REMARKS

Reexamination and reconsideration in light of the foregoing amendments to the specification and claims, and the following remarks is respectfully requested.

Claims 13-22 are pending in this application. Claims 1-12 have been canceled without prejudice or disclaimer. Claims 13, 17, 20 and 21 have been amended in accordance with suggestions made by the Examiner.

Applicants note the Examiner's consideration of the information cited in the Information Disclosure Statement filed May 12, 2003 as acknowledged in the Office Action Summary. The duplicate citations are noted.

Objection to the Specification

The specification was objected to as containing new matter in that the values 1.03 ± 0.030 and 0.07 are not seen to be obvious typographical errors. In Table I by the last amendment, the value 134 ± 0.03 was changed to 103 ± 0.03 . This was a typographical error in the last amendment and the original value " 134 ± 0.03 " should have been changed to " 1.34 ± 0.03 ." By amendment in this response, " 134 ± 0.03 " has been changed to " 1.34 ± 0.03 ." A person skilled in the art would have recognized that the original value 134 ± 0.03 was incorrect and was an obvious typographical error and should have been 1.34 ± 0.03 . The obviousness of the error becomes clear when compared to the other I_{\max} values in the Table and comparing the relationship of the I_{\max} values to the K_m^{app} , S, C and DL values in the Table. As further evidence, see Table 1 in the article in the International Search Report by M. Niculescu entitled "Redox Hydrogel-Base Amperometric Bienzyme Electrodes for Fish Freshness Monitoring," *Anal. Chem.*, 72, 1591-1597 (2000). Thus, the missing period (.) in "134" should be recognized as a typographical error.

As for typographical error with respect to “0.70” in the “DL” column of Table I, a comparison of the DL value of the “Purtrescine” analyte for the AO 67% and HRP 33% type electrode to the other DL values for other “Purtrescine” analyte for other electrodes shows that the original value is clearly out of sync. A person skilled in the art would have recognized that the period (.) in the value was misplaced and that the original value was not an aberration.

For all of the foregoing reasons, it is requested that the Examiner withdraw the holding of new matter.

Claim Objections

The Examiner objected to the language “determination of freshness biomarkers in the form of biogenic amines” in claim 13. The Examiner suggested changing the language to --determination of biogenic amines as freshness markers--. The Examiner’s suggestion has been adopted, and claim 13 has been amended accordingly.

Claim 17 was objected to because of the word “on to,” which the Examiner suggested be changed to --onto--. The Examiner’s suggestion has been adopted, and claim 17 has been amended accordingly.

The Examiner objected to the phrase “on the top” as used in the context of claim 17. The Examiner suggested amending the claim to recite that both the Type II and Type III are coated on the surface of the electrode. The Examiner’s suggestion has been adopted, and claim 17 has been amended accordingly.

The Examiner found the change in line 23 of page 12 of the specification from “metyldiater to --methyldiater-- to be confusing. The term has been changed to “mediator” as suggested by the Examiner.

Rejections Under 35 U.S.C. § 112

Claims 20 and 21 stand rejected under 35 U.S.C. 112, second paragraph, "as being incomplete for omitting essential steps, such omission amounting to a gap between the steps." The Examiner identified the missing steps as being "1) comparison of the electrical output to some standard curve for amines or for histamine," and "2) establishing a relationship between the electrical output and freshness or for histamine." Claims 20 and 21 have been amended to include these steps. By the amendment, it is believed that the rejection is overcome. Accordingly, it is respectfully requested that the rejection be reconsidered and withdrawn.

Rejection Under 35 U.S.C. § 103

Claims 13-19 and 21 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Heller et al. (WO 199323748) in view of Ohashi et al. (US 5,565,329). Claim 13, upon which claims 14-19 and 21 depend, is directed to a biosensor and requires an electrode and (i) a mono-enzyme system of an amine oxidase or (ii) a bi-enzyme system of an amine oxidase and a peroxidase. The claim further requires that the amine oxidase is a copper-containing grass pea oxidase (E.C. 1.4.3.6) and the electrode is a carbon/graphite based electrode, and whereby the amine oxidase is cross-linked to the electrode into an osmium based redox polymer. The Examiner concedes that Heller et al. "does not teach the use of grass pea-derived, copper-containing amine oxidase or the use of the biosensor for determining freshness of food by detecting the biomarkers."

The Heller et al reference relates to an electrode for the detection of hydrogen peroxide wherein the electrode comprises an electrode having a testing surface. On the surface is a transducing film. The film comprises a cross-linked redox polymer network comprising a redox

compound having multiple redox centers and a peroxidase. Heller et al. teach that a peroxidase and a second enzyme present are isolated from each other, whereas, in the present invention, an amine oxidase is coupled to a peroxidase, i.e. the two enzymes are not isolated from each other. See page 3, lines 6-11 of Applicants' specification. Accordingly, from the teachings of Heller et al., it cannot be concluded that an electrode having the construction as defined in claim 13, can give a very sensible and selective detection/determination of biomarkers in the form of biogenic amines.

Ohashi et al. does not make up for the deficiencies of Heller et al. Ohashi et al. is discussed on page 2 of the present specification. It is evident from the discussion that the prior art method disclosed by Ohashi et al. reads on a reduction in dissolved oxygen (DO) and that said method is neither very selective nor sensitive. Ohashi et al. use a copper-containing amine oxidase extracted from *Aspergillus niger*, and not amine oxidase from grass pea as required by claim 13.

The Examiner concludes that "[o]ne of ordinary skill in the art would have been motivated to modify the biosensor as taught by Heller et al. by substituting the oxidase into a copper-containing amine oxidase (EC 1.4.3.6) because copper-containing amine oxidases (EC 1.4.3.6) are useful in determining freshness of foods to due the enzyme's strong reactivity to histamine." According to the Examiner, all enzymes in EC 1.4.3.6 would have the same results because these enzymes have the same reaction mechanism.

It is respectfully submitted that this is not true since grass pea amine oxidase exhibits a higher sensitivity than other amine oxidases. The Examiner maintains that the two enzymes in Heller et al. are "kept in separate layers." However, the Examiner contends that "this does not mean

that the reactions are not coupled.” and that “the enzyme reactions are selected so that they can be coupled.” According to the Examiner:

The first enzyme on the electrode generates peroxide so that the main enzyme, peroxidase, will have a substrate to act upon. Sarcosine oxidase is a specific example of a suitable coupling first enzyme (see paragraph bridging pages 15-16).

The explanation by the Examiner fails to indicate how the enzyme reactions are selected so that they are coupled. The Examiner has described the reaction with the first enzyme to produce a peroxide, but has not explained from the teachings of the reference how this first enzyme is coupled to the second enzyme.

The Examiner further argues:

According to Expasy (<http://www.expasy.org/cgi-bin/nicezyme.pl?4.3.6>), most all of the amine oxidases have copper and topaquinone (2,4,5-hydroxyphenylalanine quinone) as their cofactors. It is not unique to grass pea copper-amine oxidase. This confirmed by Brenda (http://www.brenda.uni-koeln.de/php/result_flat.php-3?ecno=1.4.3.6). The enzymes from a variety of sources are known in the art as shown by Brenda. The grass pea, *Lathyrus sativus*, is in the same genus as *Lathyrus cicera*, for which the amine oxides has been partially purified and studied (Cogoni et al., 1989).

There does not appear to be any advantage in the selection of one amine oxidase over another for the method instantly claimed. Further the disclosure does not support the argument in the response that the enzyme from grass pea is more selective and sensitive than the enzyme from *Aspergillus*. While it is clear from the information at Brenda, that the enzyme from each species will have varying values for K_m , there is no evidence in the disclosure or the art that supports the generic argument of the response. What the instant disclosure actually says is not that the enzyme from *Aspergillus* has less selectivity and sensitivity than the enzyme from grass pea, but rather that the method or “approach is not very selective and sensitive” (page 2, lines 10-11), referring to the dissolved oxygen measurement being made in Ohashi et al. There is no advantage disclosed in the specification that the grass pea amine oxidase has over any other amine oxidase for the claimed method.

The Expasy reference relied upon by the Examiner does not disclose topaquinone. Further, the Examiner has not explained how the reference shows that “most all of the amine oxidases have copy

and topaquinione ... as their cofactors” and why this is “not unique to grass pea copper amine oxidase. The Examiner refers to Brenda and Cogoni et al. (1999), but no copies of Brenda and Cogoni et al. to make the asserted showing. The Examiner has not presented cogent scientific reasoning from Brenda, Expasy, Heller et al. and/or Ohashi et al. to show that “[t]here does not appear to be any advantage in the selection of one amine oxidase over another”

To the extent that the present rejection is based on the argument in the previous response that the enzyme from grass pea is more selective and sensitive than the enzyme from *Aspergillus niger* has not been supported by evidence. In support of the arguments, the following data from a comparative test is provided in the table below.

Enzyme	Transducer	E vs. Ag/AgCl (mV)	Selectivity	LR (μM)	DL (μM)	S (mA/M)
AO Grass Pea HRP	Graphite	-50	His 100% Put 147% Cad 132%	1-450 1-400 1-400	0.33 0.17 0.20	5.16 13.58 11.80
AO Grass Pea	Graphite	+200	His 100% Put < 1% Cad < 1%	10-200 - -	2.2 - -	0.48 - -
AO <i>Aspergillus niger</i>	Pt Paste	+600	His Put	0.17-20 0.06-200	n.a. n.a.	0.93 0.24

The comparison relates to different transducer systems, however, it should nevertheless be evident that *Aspergillus niger* AO gives an inferior sensitivity in comparison with grass pea AO (0.93 vs. 0.48) and that there is no need to use the same high potential (+200 mV in the case of grass pea AO vs. +600 mV in the case of *Aspergillus niger* AO). High voltages result in high background currents and poor selectivity due to interfering signals emanating from electrochemical interferences, HRP systems become quite different, but give on the other hand a great selectivity

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with regard to putrescine (Put) and cadaverine (Cad). Moreover, *Aspergillus niger* AO cannot be used for putrescine. In that case, peroxidase from *Micrococcus rubens* will be required.

The two constants characterizing a calibration curve obtained by means of an amperometric biosensor are given as I_{\max} and K_m^{app} . Such values are evident from the publication cited in the International Search Report, namely, M. Nichlescu et al., *supra* (see Table 1 on p. 1595). Total concentration of amine in fish expressed in histamine (His) is also evident from the publication.

For the foregoing reasons, it is respectfully requested that the rejection of claims 13-19 and 21 under 35 U.S.C. § 103(a) over Heller and Ohashi et al. be reconsidered and withdrawn.

Conclusion

It is submitted that the claims 13-22 are patentable in light of the preceding amendments and remarks set forth above. Allowance of the claims is courteously solicited.

To the extent necessary, a petition for an extension of time under 37 CFR § 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

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